Supplementary material for

"Hemoglobin mRNA changes in the frontal cortex of patients with neurodegenerative diseases"

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Figure S1. Titration of reference and hemoglobin genes expression levels in blood samples. Absolute C_T for target (*HBB* and *HBA1/2*) and two reference genes (*ACTB* and *RPL19*) of blood and brain samples are shown. Pool of blood and brain cDNA samples (n = 2 each) were prepared.

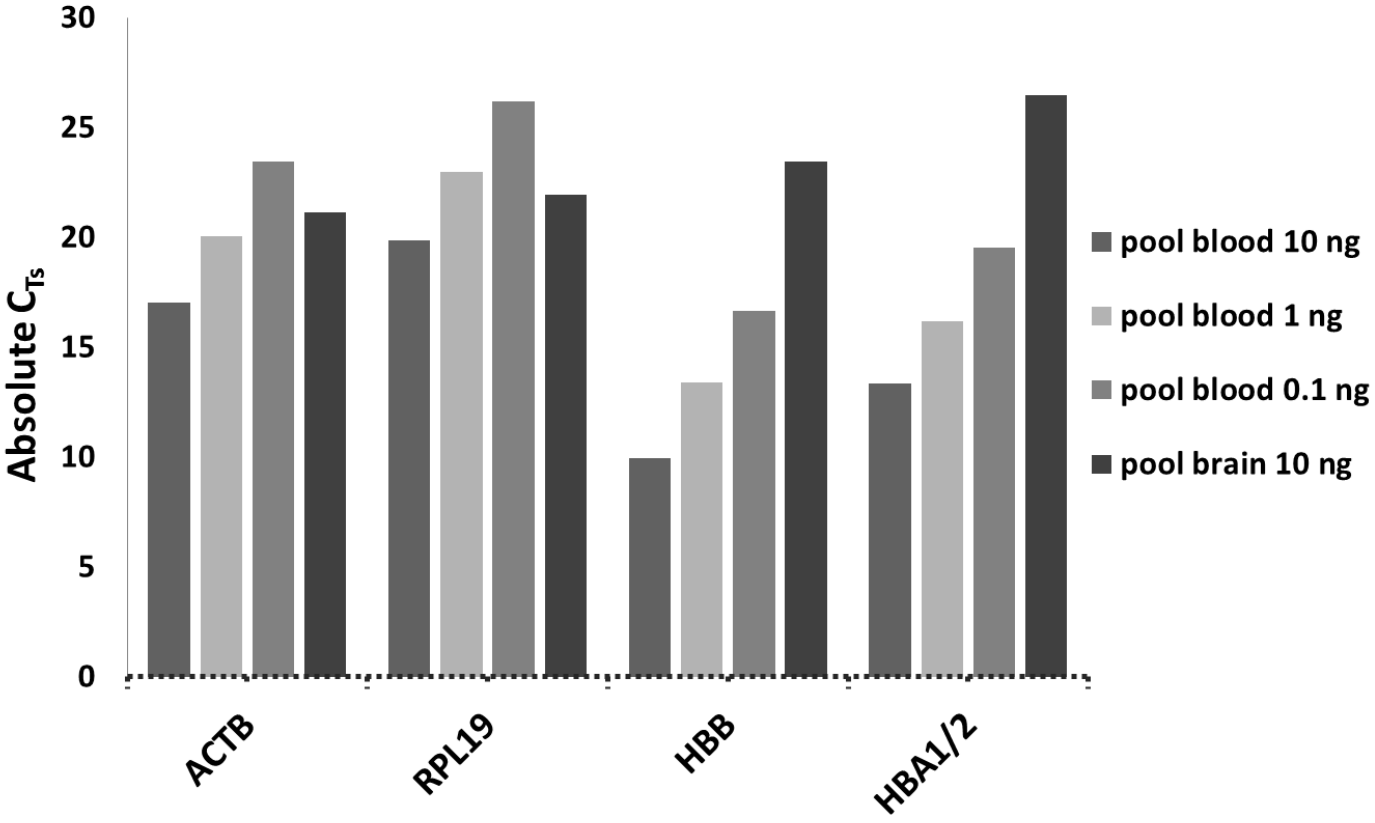

Figure S2. qPCR validation for the "blood normalization" method with blood cDNA from healthy controls. Relative expression levels of *HBB* and *HBA1/2* against *GAPDH* with and without *ALAS2* normalization in brain samples (pool of 2 AD samples) containing serially diluted amounts of blood cDNA (pool of 2 healthy controls).

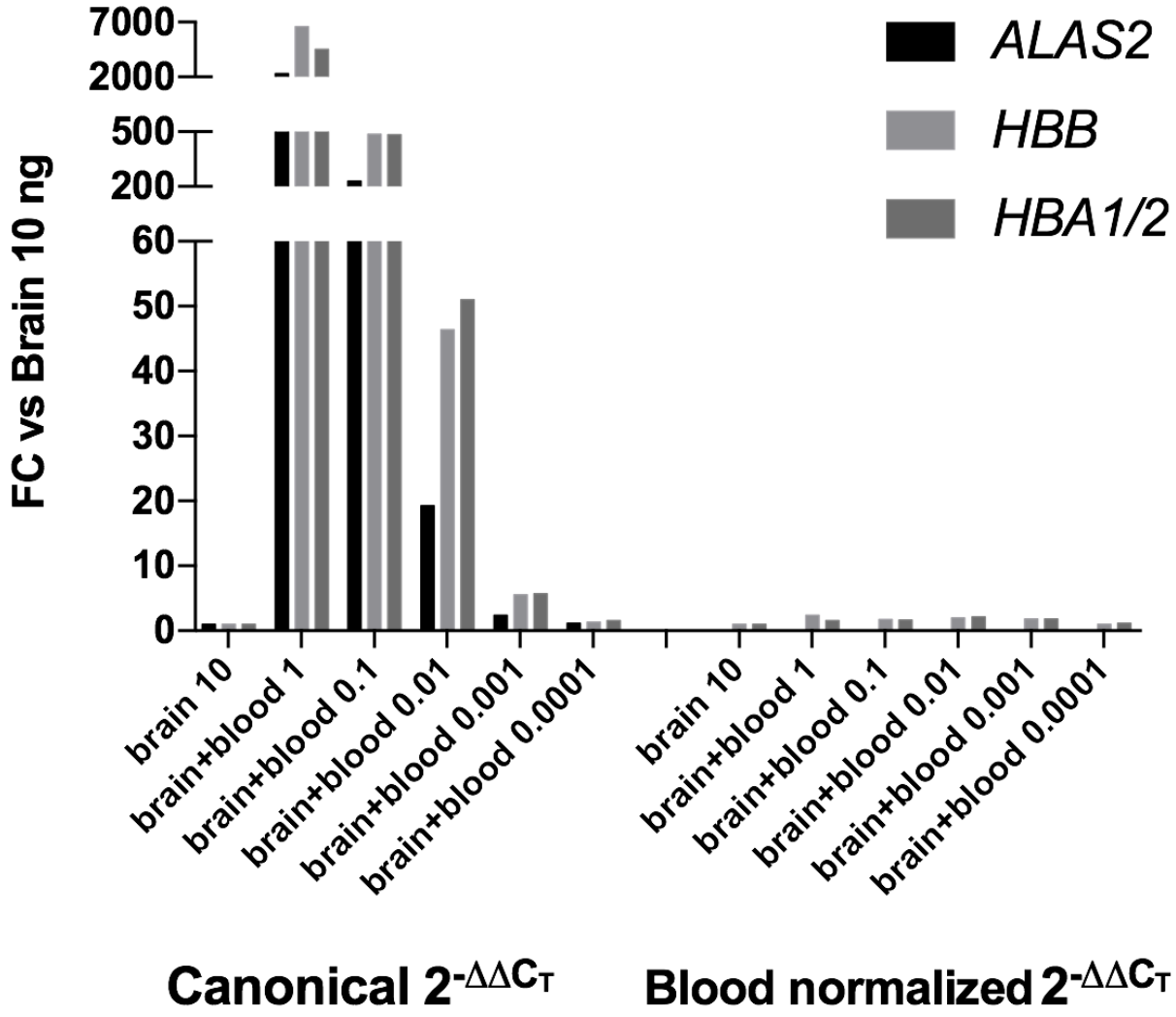

Figure S3. qPCR validation for the "blood normalization" method with blood cDNA from diseased patients. Relative expression levels of *HBB* and *HBA1/2* against *GAPDH* with and without *ALAS2* normalization in brain samples (pool of 2 AD samples) containing serially diluted amounts of blood cDNA (pool of 2 patients).

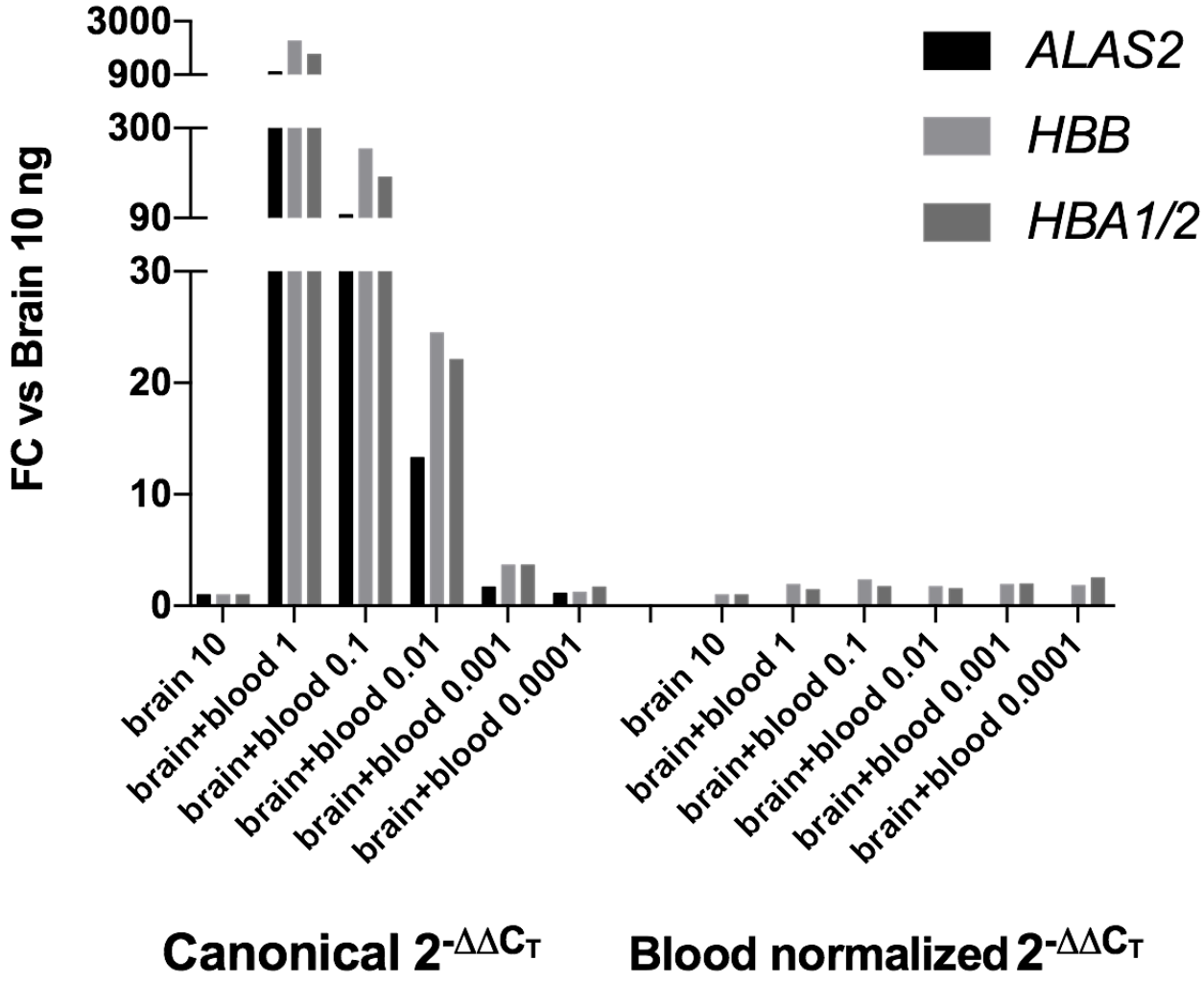

Figure S4. Blood-normalized *HBB* and *HBA1/2* expression. Relative expression levels of *HBB* and *HBA1/2* against *ACTB* and against *ALAS2* in gPrD, sCJD, vCJD, iCJD and AD patients. *=p<0.05, **=p<0.005

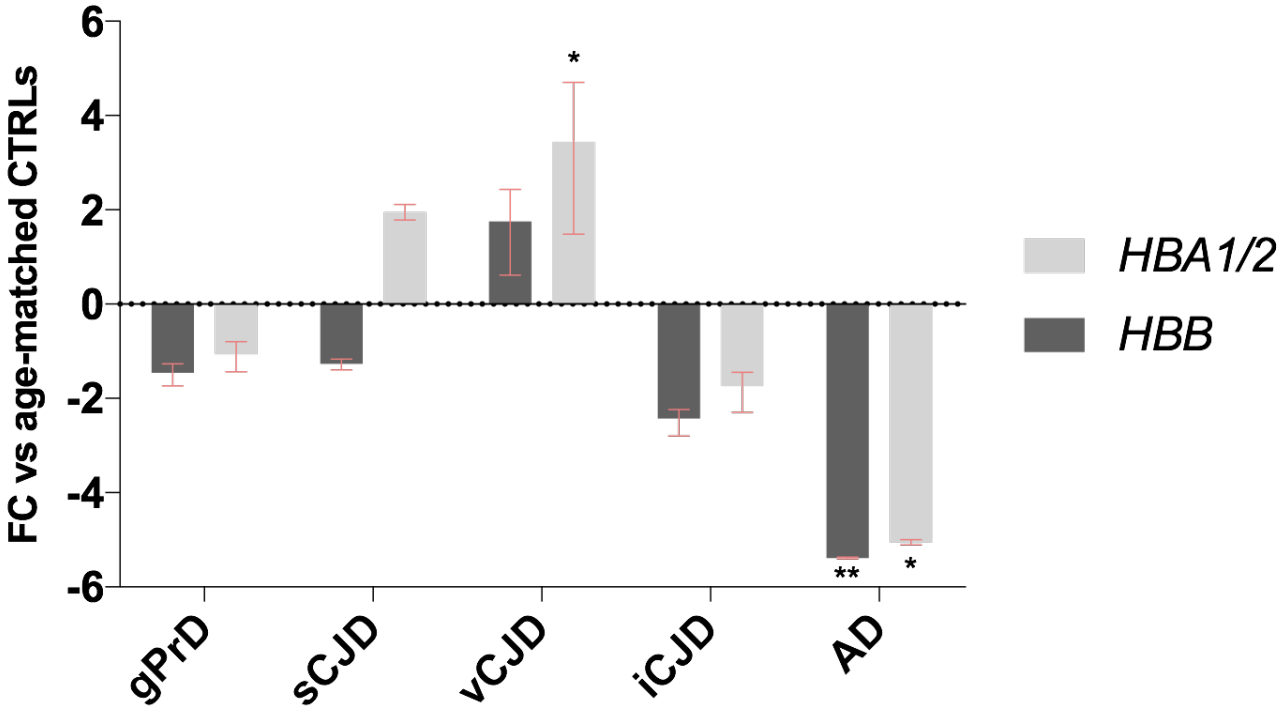

Figure S5. Blood-normalized *HBB* and *HBA1/2* expression. Relative expression levels of *HBB* and *HBA1/2* against *RPL19* and against *ALAS2* in gPrD, sCJD, vCJD, iCJD and AD patients. *=p<0.05

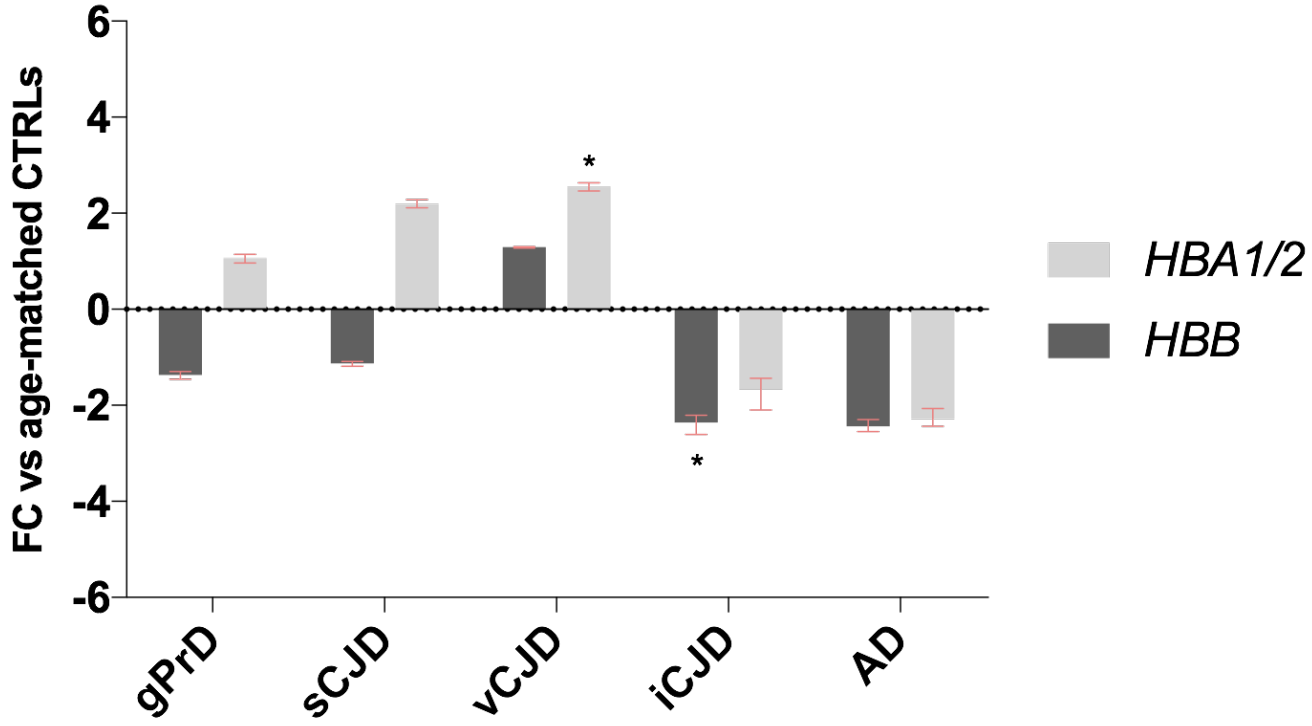

Figure S6. Blood-normalized *HBB* and *HBA1/2* expression. Relative expression levels of *HBB* and *HBA1/2* against *B2M* and against *ALAS2* in gPrD, sCJD, vCJD, iCJD and AD patients. *=p<0.05, **=p<0.01

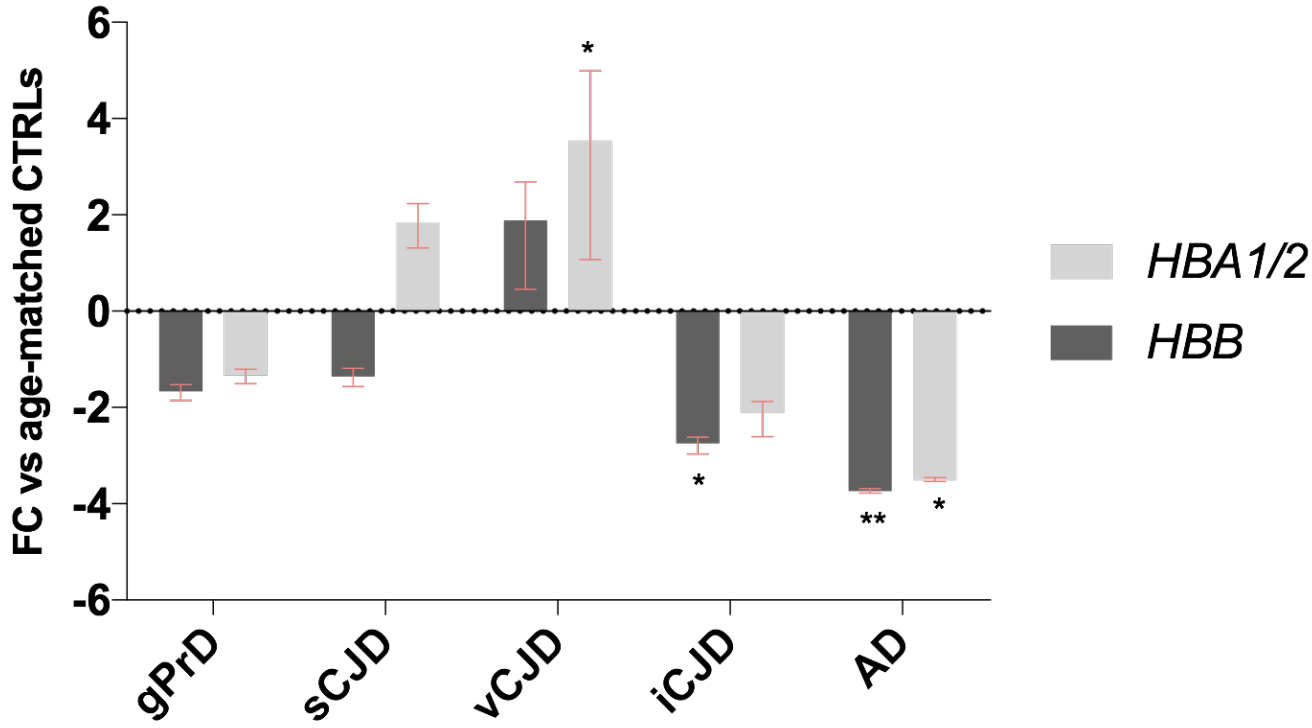

Figure S7. Comparison of mean age between AD group and related healthy controls. *n.s,* not significant

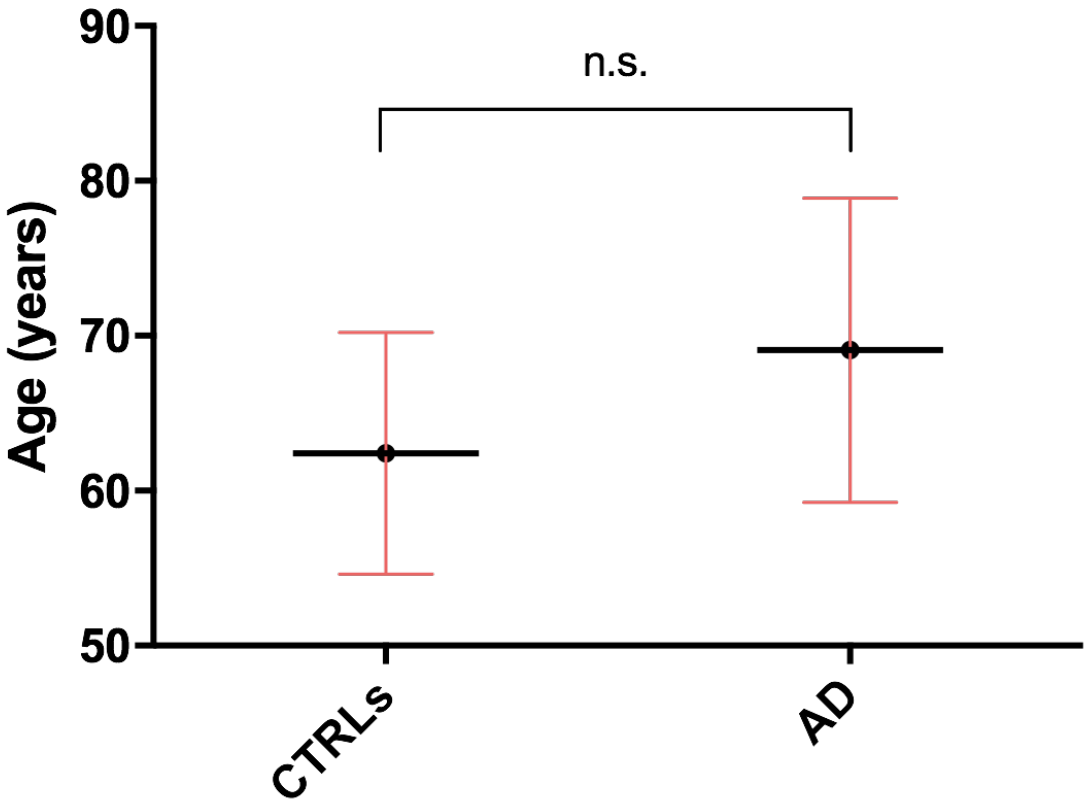

Figure S8. *HBB* and *HBA1/2* expression levels in females and males.

ΔC_T values were normalized against *GAPDH* and *ALAS2*. F female, M male

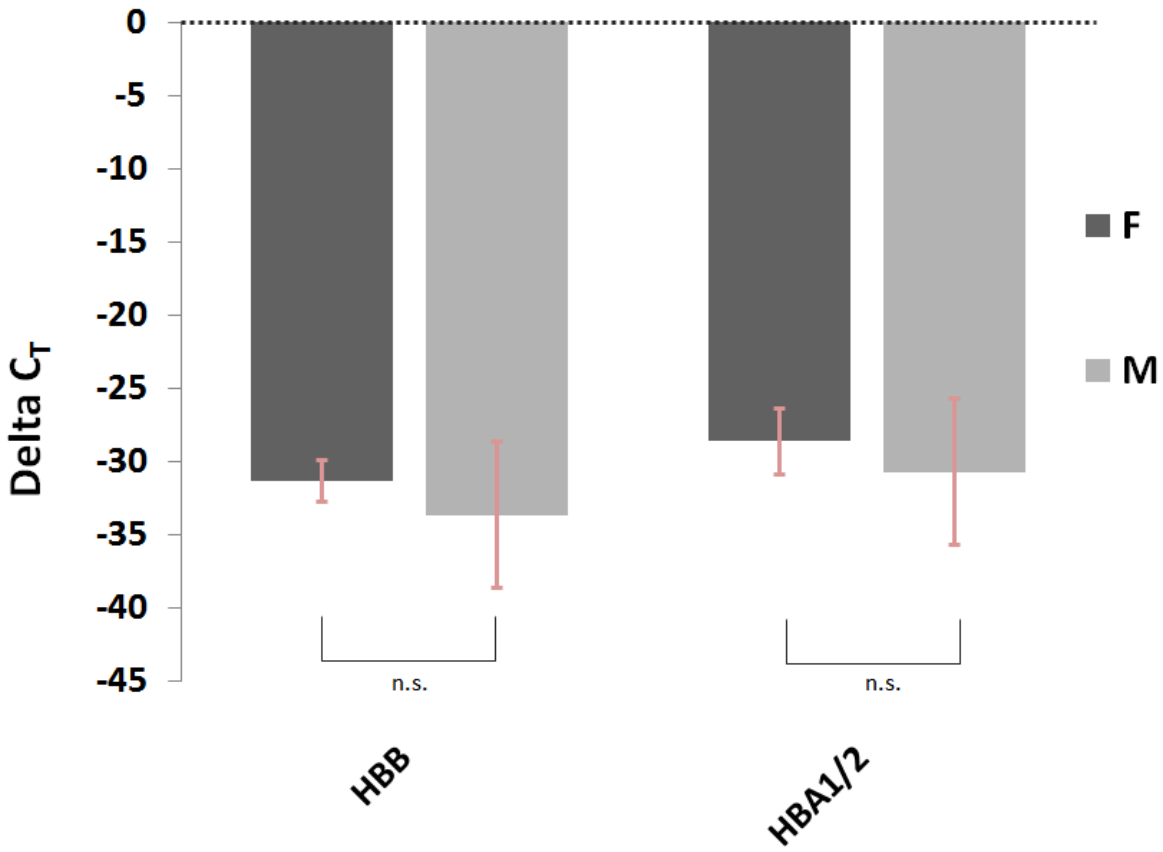

Figure S9. *HBB* and *HBA1/2* expression levels across healthy controls.
ΔC_T values were normalized against *GAPDH* and *ALAS2*. Age of each single patient is listed on the X axis.

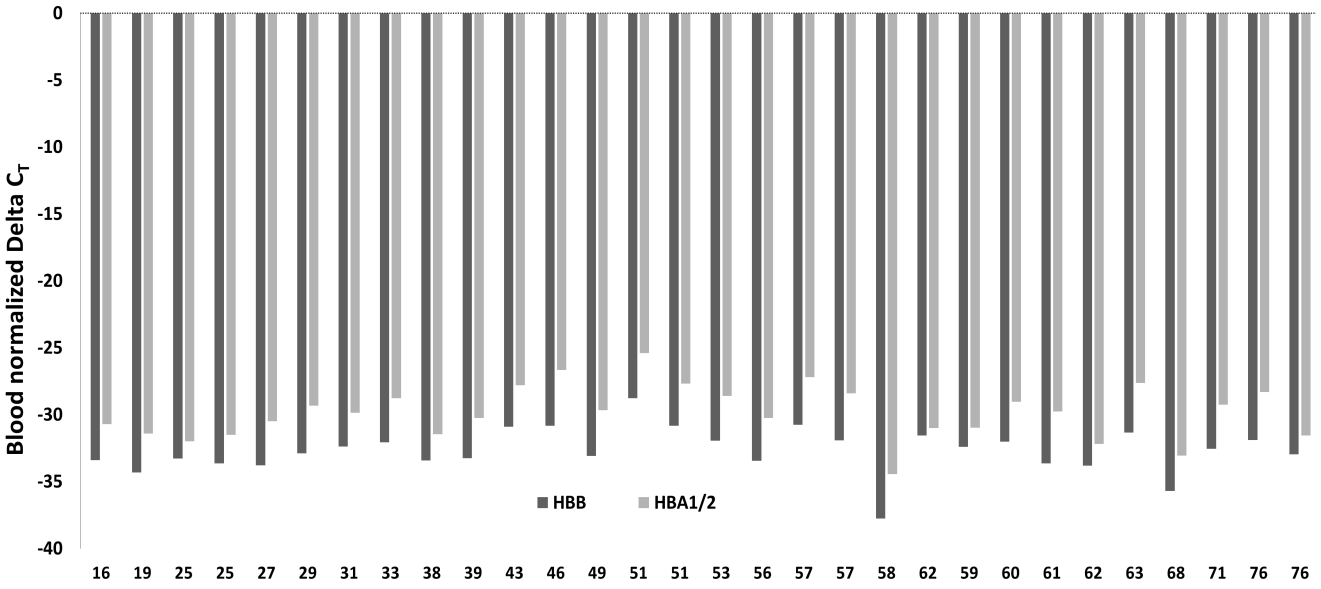

Figure S10. Hemoglobin α-chain (A, B, C) and β-chain (D, E, F) in the frontal cortex of a single sCJD case. Immunofluorescence and confocal microscopy for hemoglobin α-chain (A), β-chain and nuclei (B, E) showing increased immunoreactivity in astrocytes for α-chain hemoglobin (C merge) but not for . β-chain (F merge) Nuclei staned with DRAQ5™. Bar = 50 µm.

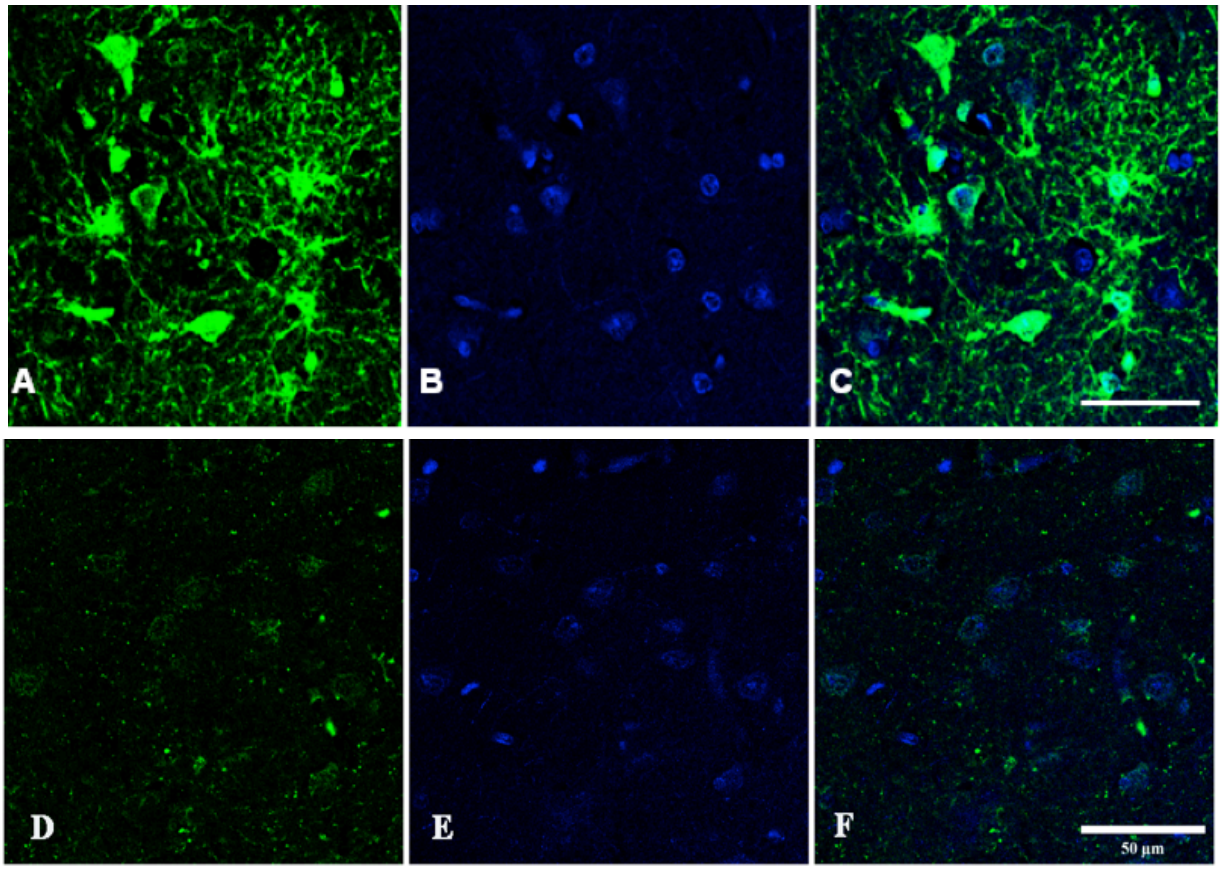

Figure S11. Double-labelling immunofluorescence in the frontal cortex of a single sCJD case.

A-C) Double-labelling immunofluorescence and confocal microscopy showing haemoglobin A (green, A) and glial fibrillary acidic protein (red, B) in the cerebral cortex in one case with Creutzfeldt-Jakob's disease. Haemoglobin is found in many astrocytes (arrows, C merge). D-F) Double-labeling immunofluorescence and confocal microscopy showing haemoglobin B (green, D) and NeuN (red, E). Haemoglobin is found in most if not all neurons (arrowheads, F merge). Paraffin sections; Bar = 40 microns.

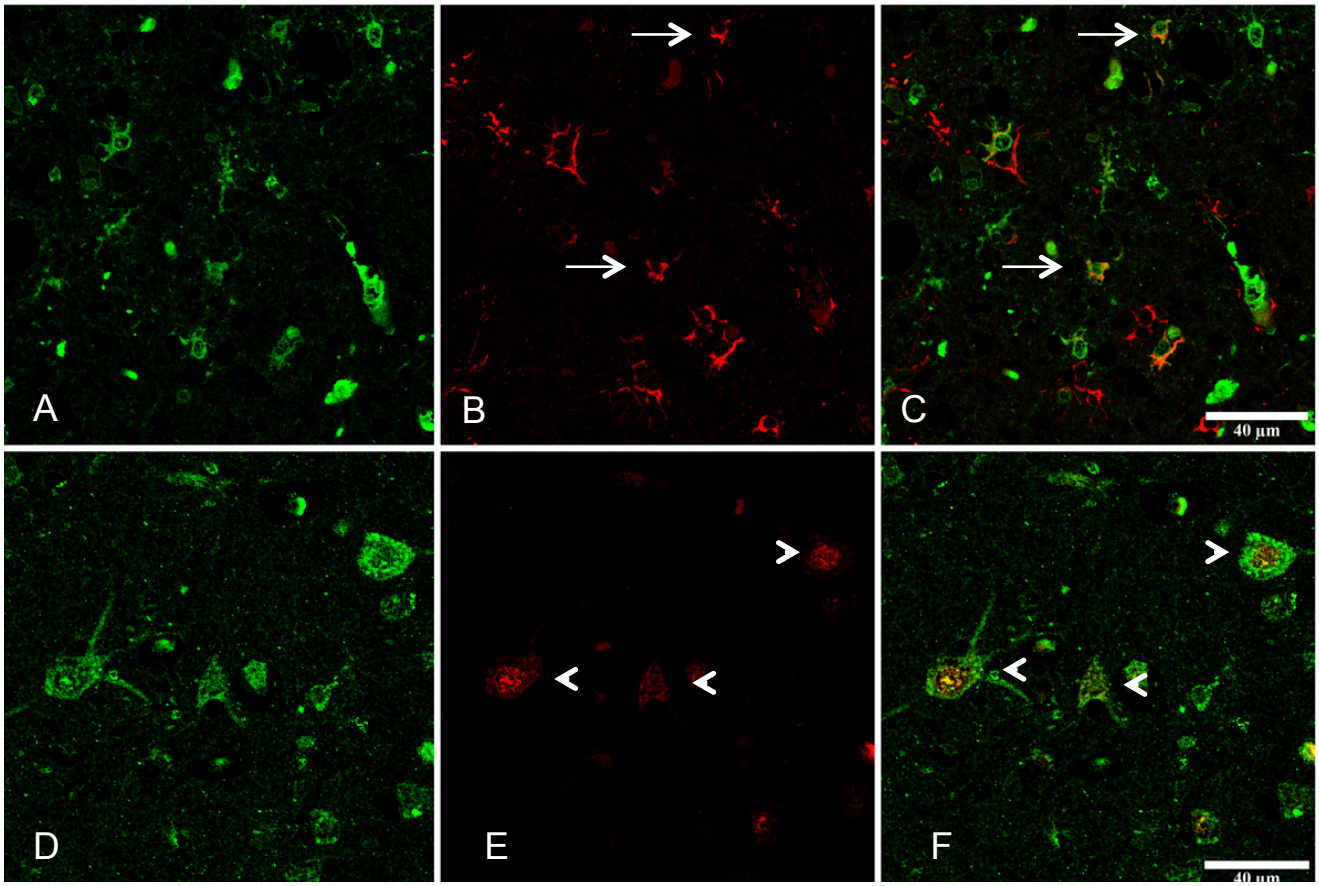

Table S1. List of AD and healthy controls. F female, M male, BS Braak stage

AD	SEX	AGE	BRAAK STAGE	CTRLs	SEX	AGE
A10/46	M	74	AD, BS II	4176	M	51
A11/13	M	70	AD, BS I	15221	M	53
A10/64	M	86	AD, BS II	3783	M	56
A10/45	M	67	AD, BS I	24781	M	57
A10/6	M	57	AD, BS II	18391	M	58
A11/75	M	61	AD, BS I	7628	M	60
A10/98	F	73	AD, BS I	22612	M	61
A11/51	M	58	AD, BS I	18407	M	62
A10/27	M	68	AD, BS I	20121	M	63
A10/77	M	65	AD, BS II	13410	M	68
A11/55	M	60	AD, BS II	14395	F	71
A10/34	M	64	AD, BS I	9508	M	76
1677	M	90	AD, BS III	1656	F	62
1721	M	74	AD, BS I	17/14	M	76